02-29-00

Express Mail Label No. EM322760718US

UTILITY PATENT APPLICATION TRANSMITTAL
(Large Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No. FUJ2-AZ72a
Total Pages in this Submission

jc639 U.S. PTO 02/24/00

TO THE ASSISTANT COMMISSIONER FOR PATENTS
Box Patent Application
Washington, D.C. 20231

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for an invention entitled:

IMMUNOASSAY METHOD FOR LYSED WHOLE BLOOD

jc511 U.S. PTO 09/511824 02/24/00

and invented by:

Yasuo Yamao and Narihiro Oku

If a CONTINUATION APPLICATION, *check appropriate box and supply the requisite information:*

☐ **Continuation** ☒ **Divisional** ☐ **Continuation-in-part (CIP)** of prior application No.: **08/914,039**

Which is a:

☐ **Continuation** ☐ **Divisional** ☐ **Continuation-in-part (CIP)** of prior application No.: _____

Which is a:

☐ **Continuation** ☐ **Divisional** ☐ **Continuation-in-part (CIP)** of prior application No.: _____

Enclosed are:

Application Elements

1. ☒ Filing fee as calculated and transmitted as described below
2. ☒ Specification having **thirteen (13)** pages and including the following:
   - a. ☒ Descriptive Title of the Invention
   - b. ☐ Cross References to Related Applications *(if applicable)*
   - c. ☐ Statement Regarding Federally-sponsored Research/Development *(if applicable)*
   - d. ☐ Reference to Microfiche Appendix *(if applicable)*
   - e. ☒ Background of the Invention
   - f. ☒ Brief Summary of the Invention
   - g. ☒ Brief Description of the Drawings *(if drawings filed)*
   - h. ☒ Detailed Description
   - i. ☒ Claim(s) as Classified Below
   - j. ☒ Abstract of the Disclosure

UTILITY PATENT APPLICATION TRANSMITTAL
(Large Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No. NAK1-BJ92
Total Pages in this Submission

Application Elements (Continued)

3. ☒ Drawing(s) *(when necessary as prescribed by 35 USC 113)*

   a. ☒ Formal Number of Sheets **Seven (7)**

   b. ☐ Informal Number of Sheets _____

4. ☒ Oath or Declaration

   a. ☐ Newly executed *(original or copy)* ☐ Unexecuted

   b. ☒ Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional application only)*

   c. ☒ With Power of Attorney ☐ Without Power of Attorney

   d. ☐ *DELETION OF INVENTOR(S)*
   Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. 1.63(d)(2) and 1.33(b).

5. ☐ Incorporation By Reference *(usable if Box 4b is checked)*
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

6. ☐ Computer Program in Microfiche *(Appendix)*

7. ☐ Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all must be included)*

   a. ☐ Paper Copy

   b. ☐ Computer Readable Copy *(identical to computer copy)*

   c. ☐ Statement Verifying Identical Paper and Computer Readable Copy

Accompanying Application Parts

8. ☐ Assignment Papers *(cover sheet & document(s))*

9. ☐ 37 CFR 3.73(B) Statement *(when there is an assignee)*

10. ☐ English Translation Document *(if applicable)*

11. ☒ Information Disclosure Statement/PTO-1449 ☐ Copies of IDS Citations

12. ☒ Preliminary Amendment

13. ☒ Acknowledgment postcard

14. ☒ Certificate of Mailing

   ☐ First Class ☒ Express Mail *(Specify Label No.):* **EM322760718US**

UTILITY PATENT APPLICATION TRANSMITTAL
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Docket No. NAK1-BJ82
Total Pages in this Submission

Accompanying Application Parts (Continued)

15. ☐ Certified Copy of Priority Document(s) *(if foreign priority is claimed)*

16. ☐ Additional Enclosures *(please identify below):*

Fee Calculation and Transmittal

CLAIMS AS FILED

For	#Filed	#Allowed	#Extra		Rate	Fee
Total Claims	5	- 20 =	0	x	$18.00	$0.00
Indep. Claims	2	- 3 =	0	x	$78.00	$0.00
Multiple Dependent Claims (check if applicable) ☐						$0.00
					BASIC FEE	$690.00
OTHER FEE *(specify purpose)* _____						
					TOTAL FILING FEE	$690.00

☒ A check in the amount of **$690.00** to cover the filing fee is enclosed.

☒ The Commissioner is hereby authorized to charge and credit Deposit Account No. **16-2462** as described below. A duplicate copy of this sheet is enclosed.

- ☐ Charge the amount of as filing fee.
- ☒ Credit any overpayment.
- ☒ Charge any additional filing fees required under 37 C.F.R. 1.16 and 1.17.
- ☐ Charge the issue fee set in 37 C.F.R. 1.18 at the mailing of the Notice of Allowance, pursuant to 37 C.F.R. 1.311(b).

Signature

Dated: **February 24, 2000**

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cc:

FUJ2-AZ72a

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Previous Examiner: C. Spiegel
Yasuo Yamao et al.	Group Art Unit: 1641
Serial No.:	
Filed:	February 24, 2000
For: IMMUNOASSAY METHOD FOR LYSED WHOLE BLOOD	Irvine, California 92614

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to an examination on the merits of the above-identified application, please enter the following amendments:

IN THE SPECIFICATION:

Page 1, between lines 2 and 3, insert --This is a divisional application of U.S. Serial No. 08/914,039, filed on July 28, 1997.--.

Page 2, line 4, delete "lyzed" and insert --lysed--;

Page 5, in Table 1, line 14, delete "Triton" and insert --TRITON--;

line 14, delete "Tween" and insert --TWEEN--;

line 20, delete "Brijj" and insert --BRIJ--;

Page 6, line 10, delete "lyze" and insert --lyse--;

line 13, after "computer" insert --and 8 is a dislplay.";

line 15, delete "non-lyzed" and insert --non-lysed--;

line 23, delete "lyze" and insert --lyse--;

Page 7, line 9, delete "lyze" and insert --lyse--;

line 10, delete "lysed" and insert --lysed--;

line 12, delete "lyzing" and insert --lysing--;

line 17, delete "lyze" and insert --lyse--;

line 17, delete "lyzed" and insert --lysed--;

line 22, delete "lyze" and insert --lysed--;

line 26, after "CRP" insert --(C-reactive protein)--;

Page 8, line 3, delete "[sic.]".

IN THE CLAIMS:

Please cancel Claims 1-7 without prejudice.

Please add the following newly drafted Claims 8-12:

8. An immunoassay system comprising:

means for lysing whole blood with a hemolysis reagent;

means for reacting antigens in the whole blood to an agglutination reaction with insoluble carriers onto which antibodies specifically reacting with the antigens in the whole blood have been immobilized; and

means for measuring the resulting agglutination mixture for a change in its absorbance by irradiation with light of a wavelength substantially free from absorption by

both hemoglobin and the hemolysis reagent, including means for determining the hematocrit % of the samples, and means for calculating a plasma concentration with the hematocrit by the following equation:

$$A' = A \times 100/(100 - \text{hematocrit \%})$$

where A is the absorbance, and A' is the corrected absorbance assuming that a plasma component in the sample is 100%.

9. An immunoassay method of quantifying a predetermined antigen in a sample of whole blood, comprising the steps of:

providing a sample of the whole blood;

adding a hemolysis reagent and a latex reagent directly to the sample of the whole blood without any pre-treatment of the whole blood;

hemolysing the whole blood sample with the hemolysis reagent to hemolyse the blood corpuscles;

reacting the hemolysed whole blood sample in an agglutination reaction to form a reaction mixture wherein a predetermined antigen in the hemolysed whole blood sample specifically reacts with an antibody immobilized onto an insoluble carrier;

irradiating the reaction products in the sample with radiation which include a wavelength range which is substantially free from absorption by both hemoglobin and the hemolysis reagent; and

measuring only in a wavelength range which is substantially free from absorption by both hemoglobin and the hemolysis reagent, an absorbance of the incident radiation through the reaction mixture to determine the quantity of antigens in the sample.

10. The immunoassay method of Claim 9, wherein the step of measuring is performed by determining the hematocrit percentage (%); and

calculating a plasma concentration with the hematocrit % as follows:

$$A' + AX\ 100/(100 - \text{hematocrit \%})$$

where A is the absorbance, and A' is the corrected absorbance assuming that the plasma component in the sample is 100 %.

11. The immunoassay method of Claim 10, wherein the step of hemolysing is performed with a saponin aqueous solution.

12. The immunoassay method of Claim 11, wherein the measuring step is performed with the use of an erythrocyte counter.

REMARKS

This is a divisional application of U.S. Serial No. 08/914,039. It is requested that the references cited in the prosecution of the parent application be considered in the prosecution of this divisional application, and accordingly copies of the references cited in the parent application are attached hereto with a form PTO 1449. The relevance of those references was set forth in the prosecution of U.S. Serial No.08/914,039.

If the Examiner believes that a telephone interview will help further the prosecution of this case, he is respectfully requested to contact the undersigned attorney at the listed telephone number.

Very truly yours,

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PATENT APPLICATION

IMMUNOASSAY METHOD FOR LYZED WHOLE BLOOD

Yasuo Yamao
Narihiro Oku

IMMUNOASSAY METHOD

FOR LYZED WHOLE BLOOD

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to an immunoassay method and in particular to an immunoassay method for use with lyzed whole blood in which antibodies or antigens in a sample are subjected to agglutination reaction with insoluble carriers onto which antigens or antibodies specifically reacting with the antibodies or antigens in the sample have been immobilized. The resulting agglutination mixture is irradiated with near infrared or infrared rays to determine its change in absorbance or its change in scattered light.

2. Description of Related Art

Japanese Patent Publication No. 11575/1983 discloses a prior art method which comprises antigen-antibody reaction between antigen- or antibody-immobilized insoluble carriers and antibodies or antigens in a humor sample, then irradiating the reaction mixture with light with a wavelength of 600 to 2400 nm and measuring the increase in its absorbance. By virtue of its usefulness, this method has become the mainstream of immunoassay method at present as a so-called latex immunoturbidimetry.

However, the measurement sample used in said measurement method is water, serum, urine, saline etc. In addition, matters that require attention in general blood taking for clinical examination are that hemolysis should be avoided to a maximum degree and blood should be separated into serum and plasma as rapidly as possible. The reasons for this include the effect of hemolysis on optical measurement, the incoming and outgoing of substances such as Na, K, C1 through blood membrane, the effect of

movement by blood corpuscles metabolism (i.e., transfer of lactic acid and pyruvic acid to serum by glycolysis) and the effect of the difference in concentration of the object component in blood corpuscles and in serum.

For the above reasons, blood obtained from a subject should be a sample separated into serum or plasma by centrifugation. Therefore, such pretreatment by centrifugation may not be carried out in small or private laboratories or urgent laboratories other than central laboratories in large or middle hospitals where a large amount of blood can be dealt with, and therefore the above method is not necessarily universal.

Under the circumstances of such general whole blood handling, in the field of clinical examination there is no accurate and quantitative measurement method in which whole blood can be directly used as a measurement sample without separating it into serum and plasma. Further, the measurement of blood using optical means without hemolysis is inappropriate because of high turbidity caused by erythrocyte.

OBJECTS AND SUMMARY OF THE INVENTION

In view of the foregoing, the present invention was made, and a first object is to provide an immunoassay method which can be effected easily in a short time even without pretreating blood by, e.g., a centrifuge, and a second object is to provide an immunoassay method using whole blood directly as a sample in which blood corpuscles are subjected intentionally to forcible hemolysis in a manner not to affect immunoreaction so that accurate data can be obtained in combination with various quantitative measurement reagents.

As a result of their research, the present inventors unexpectedly found that antigens or antibodies in whole blood can be determined by subjecting whole blood intentionally to forcible hemolysis in a manner not to affect agglutination reaction, as opposed to the fixed concept of general blood taking for clinical examination, that is, hemolysis should be avoided to a maximum degree and blood should be separated into serum and plasma as rapidly as possible.

To achieve the first object, the present invention comprises an immunoassay method in which antibodies or antigens in a sample are subjected to agglutination

reaction with insoluble carriers onto which antigens or antibodies specifically reacting with the antibodies or antigens in the sample have been immobilized and the resulting agglutination mixture is determined for the change in its absorbance or in its scattered light by irradiation with light, wherein said sample is whole blood and the whole blood is forcibly lyzed.

In this case, the means of forcible hemolysis can include:

(1) mixing whole blood with a low osmotic solution;

(2) mixing blood with a solution of saponins for hemolysis;

(3) freezing and thawing whole blood; and

(4) ultrasonicating whole blood.

Alternatively, saponins for hemolysis may be incorporated into an insoluble particle suspension reagent onto which antibody or antigen specifically reacting with antigen or antibody has been immobilized.

To achieve the second object, the immunoassay method according comprises the step of subjecting antibodies or antigens in whole blood as a sample to agglutination reaction with an insoluble particle suspension reagent onto which antigens or antibodies specifically reacting with the antibodies or antigens in the whole blood have been immobilized, the step of determining the resulting agglutination mixture for the change in its absorbance or in its scattered light by irradiation with light, and the step of calculating the hematocrit % of as follows by the sample:

$$A' = A \times 100/(100 - \text{hematocrit } \%)$$

where A is the absorbance or its change or the strength of light scattering or its change actually determined, and A' is the corrected absorbance or its change or the strength of light scattering or its change assuming that the plasma component in the sample is 100%.

According to the first object, the following effects are achieved:

(1) By using a whole blood sample directly in the measurement procedure without subjecting it to pretreatment such as centrifugation etc., measurement time can be shortened, measurement costs can be reduced, and a measurement operation can be simplified. Because centrifugation is not required, the costs for a centrifuge or centrifuge tube, the operation for transferring a sample to a centrifuge tube, the time for

centrifugation can be eliminated, and the opportunity for an operator to come into contact with blood can be decreased whereby the danger of infection can be significantly reduced.

(2) By subjecting blood corpuscles in whole blood to forcible hemolysis in a manner not to cause any effect on antigen-antibody reaction, the method can be combined with a measurement kit using general latex immunoturbidimetry whereby accurate measurement data can be obtain while a wide variety of applications is made feasible.

(3) By incorporating a hemolysis reagent into a latex reagent, the constitution of the measurement apparatus can be simplified and the measurement time can be reduced.

According to the present invention, accurate data can be obtained by hematocrit correction.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a drawing showing an example of the cell used in the method of the present invention.

Fig. 2 is a schematic drawing showing the constitution of the spectrophotometer used in the method of the present invention.

Fig. 3 is a drawing showing an example of the freezing cell holder used in the method of the present invention.

Fig. 4 is a drawing showing an example of the ultrasonication nozzle used in the method of the present invention.

Fig. 5 is an absorption spectrum at 300 to 1000 nm of whole blood having been subjected to hemolysis with aqueous solutions of various hemolysis reagents

Fig. 6 is a drawing showing hemolysism reaction time course at 800 nm of whole blood having been subjected to hemolysis with aqueous solutions of various hemolysis reagents.

Fig. 7 is a calibration curve of change in absorbance per minute as a function of CRP concentration obtained when CRP measurement is carried out.

Fig. 8 is a graph showing a correlation between a whole blood sample and a plasma sample when no hematocrit correction is carried out.

Fig. 9 is a graph showing a correlation between a whole blood sample and a plasma sample when hematocrit correction is carried out.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Hereinafter, the present invention is described in detail by reference to Examples.

The reagents examined in the Example are shown in Table 1 below. The symbols "a" to "g" in Table, 1 are identical with those in FIGS. 5 to 7.

Table 1

		Hemolysis Method	Content (W/V%)*	Absorbance (800 nm)	ΔAbsorbance/ min
Hemolysis Reagent Aqueous Solution	a	Pure Water (Deionized Water		0.204	0.003
	b	Saponin Aqueous Solution	0.5	0.147	0.000
	c	Triton X-100 (Non-Ionic Surface Active Agent)	0.5	0.146	0.000
	d	Tween-20 (Non-Ionic Surface Active Agent)	0.5	0.298	0.055
	e	Brij-35 (Non-Ionic Surface Active Agent)	0.5	2.312	
	f	Sodium Lauryl Sulfate (Anionic Surface Active Agent)	0.5	0.176	0.000
	g	Benzalkonium Chloride (Cationic Surface Active Agent)	0.5	0.139	0.000
	h	Freezing Hemolysis		0.163	0.000
	i	Ultrasonication Nozzle Hemolysis		0.196	0.001
	j	Physiological Saline		3.000	

*W/V% = weight/volume %

[Example 1] Hemolysis by Hemolysis Reagents

0.04 ml of human whole blood collected in a usual manner using an EDTA-2K anticoagulant was placed in quartz cell 5 with a cell length of 5 mm as shown in FIG. 1. 2.0 ml of each of Hemolysis regent aqueous solutions "a" to "g" shown in Table 1 was added to it and monitored for its absorption spectrum at 300 to 1000 nm (see FIG. 5) in spectrophotometer 1 (e.g., U-3410, produced by Hitachi, Ltd., Japan), for its Hemolysis reaction time course at 800 nm (see FIG 6), for its absorbance 5 minutes after the reaction was initiated and for the change in its absorbance at 800 nm for 1 minute between 4 and 5 minutes after the reaction was initiated (see Table 1) to examine the ability of each hemolysis reagent to lyze blood.

In FIG. 2, 2 is a halogen lamp as a light source emitting irradiation light L, e.g., near infrared or infrared rays; 3 is a condensing lens; 4 is a diffraction grating; 6 is an amplifier; and 7 is an arithmetic and recording device such as computer. "S" is whole blood having been subjected to hemolysis treatment as a sample accommodated in cell 5.

As shown in FIG. 5, non-lyzed blood treated with the reagent "j" (physiological saline) showed an absorbance of 2.5 or more due to its turbidity at the shown wavelengths, resulting in affecting the optical detection of latex agglutination reaction. On the other hand, it was found that as shown in FIG. 5, such turbidity as described above disappeared by use of the reagent "a" (pure water) and the reagent "b" (saponin aqueous solution) so that the degree of agglutination of latex can be determined. As can be seen from Table 1 and FIG. 6, the reagent "a" (pure water), the reagent "b" (saponin), the reagent "o" (Triton X-100), the reagent "f" (sodium lauryl sulfate), and reagent "g" (benzalkonium chloride) have the ability to lyze whole blood in a short time.

[Example 2] Hemolysis by Freezing

FIG. 3 shows one embodiment of freezing cell holder 9 for use in lyzing whole blood, which can receive and maintain cell 5 in it and includes a Peltier element 12 (a product of, e.g., Merukoa Co., Ltd.) attached to cell block 11 made of aluminum provided with photometric window 10. 13 is a power source for applying a direct current as necessary to Peltier element 12, and L is a near infrared or infrared ray from the power source 2.

0.04 ml of human whole blood collected in a usual manner using an EDTA-2K anticoagulant was accommodated in cell 5 placed in freezing cell holder 9 as shown in FIG. 3, and the human whole blood was completely frozen by applying an electric current to the Peltier element 12 for 10 minutes in a predetermined direction. Thereafter, the frozen human whole blood was thawed by applying an electric current in the reverse direction to the Peltier element 12, then diluted with 2.0 ml physiological saline, and examined for its absorbance 5 minutes after the reaction was initiated and for the change in its absorbance at 800 nm for 1 minute between 4 and 5 minutes after the reaction was initiated (see Table 1) to determine the ability of the hemolysis reagent to lyze blood. As can be seen in "h" in Table 1, human whole blood can be lyzed by freezing and thawing.

[Example 3] Hemolysis by Ultrasonication

FIG. 4 shows one embodiment of ultrasonic nozzle 14 for use in lyzing whole blood, which includes ultrasonic radiator 16 attached to stainless steel nozzle 15, and 17 is a radiator circuit, and 18 is a suction syringe.

0.04 ml of human whole blood obtained in a usual manner using an EDTA-2K anticoagulant was suctioned into nozzle 15, and the ultrasonic radiator 16 was run for 5 minutes to lyze human whole blood B in nozzle 15. Thereafter, the lyzed human whole blood B was accommodated in nozzle 15, then diluted with 2.0 ml physiological saline, and examined in spectrophotometer 1 for its absorption at 800 nm 5 minutes after the reaction was initiated and for the change in its absorbance for 1 minute between 4 to 5 minutes after the reaction was initiated (see Table 1) to determine the ability of the hemolysis reagent to lyze blood. As can be seen from symbol "i" in Table 1, human whole blood can be lyzed by ultrasonicating it.

[Example 4] CRP Measurement Method 1

1) Preparation of Anti-CRP Antibody Sensitized Latex Solution

An about 10 mg/ml anti-human CRP rabbit antibody solution (pH 7.5, 100 mmol/l Tris-HCl buffer, 0.1% sodium amide) was added to 10 ml polystyrene latex with an average particle diameter of 0.2 μm (produced by, e.g., Japan Synthetic Rubber Co., Ltd., Japan; 10% solid content), and the mixture was allowed to stand at 30 °C one whole day and night and then centrifuged at 3600 rpm to give precipitates. 0.2 W/V %

bovine serum albumin, pH 8.5, 100 mmol/l Tris-HCl buffer was added to the precipitates to prepare an anti-CRP antibody sensitized latex solution.

2) CRP Measurement Method

0.04 ml of human whole blood collected in a usual manner using an EDTA-2K anticoagulant was placed in cell 1, and 0.5 ml of each of hemolysis regent aqueous solutions "a" to "g" shown in Table 1 was added to it, and the mixture was incubated at 37°C for 3 minutes, and 1.5 ml of the anti-human CRP antibody sensitized latex suspension prepared in item 1) above was added to it, and the change in its absorbance at 800 nm for 1 minute between 4 and 5 minutes after the reaction was initiated was determined.

Separately, a calibration curve of the above sample was prepared using a commercial latex immunoturbidimetry CRP measurement kit intended for use in examining plasma as its sample. FIG. 3 [sic.] shows a calibration curve prepared using the results obtained in the above CRP measurement, and a calibration curve excellent in sensitivity as shown in symbols "a" and "b" in the figure was obtained using whole blood lyzed forcibly with pure water "an, saponin aqueous solution "b" etc. However, the results indicated that surface active agents "c" to "g" inhibit agglutination reaction and are thus not suitable for immunoreaction, as shown in the symbols "c" to "g" in the figure.

[Example 5] CRP Measurement Method Using Blood Sample Lyzed by Freezing or Ultrasonication

The operation of dilution with physiological saline after hemolysis in Example 2 or 3 was replaced by addition of 2.0 ml of the anti-human CRP antibody sensitized latex suspension prepared in Example 4, and a change in absorbance at 800 nm for 1 minute between 4 to 5 minutes after the reaction was initiated was determined in spectrophotometer 1.

Separately, a calibration curve of the above sample was prepared using a commercial latex immunoturbidimetry CRP measurement kit intended for use in examining serum or plasma as its sample. As shown in symbols "h" and "i" in FIG. 7, a calibration curve excellent in sensitivity was obtained.

[Example 6] CRP Measurement Method 2.

The same measurement method as in Example 4 was used except that a commercial latex immunoturbidimetry CRP measurement kit was used in place of the anti-human CRP antibody sensitized latex suspension used in Example 4 and 0.5 w/v S saponin aqueous solution was used as a hemolysis reagent in order to determine a change in absorbance at 800 nm for 1 minute between 4 to 5 minutes after the reaction was initiated. A comparison between a calibration curve (n = 40) using measurement values of whole blood as a sample determined according to the present invention and a calibration curve using measurement values of serum as a sample determined according to a general method indicated good correlation as shown in FIG. 8.

[Example 7] Hematocrit Correction

To correct the measurement values obtained according to the present invention in Example 6, the whole blood was simultaneously determined for its hematocrit value by means of an erythrocyte counter (e.g., LC-240A manufactured by Horiba Seisakusho K.K., Japan) using the following formula (1):

$$A' = A \times 100/(100 - \text{hematocrit \%}) \qquad (1)$$

where A is the actually determined change in absorbance, and A' is its corrected change assuming that the plasma component in the sample is 100%. A comparison (n = 40) between the measurement values thus corrected and the measurement values determined in a general method using serum as a sample indicated further improved correlation as compared with that of Example 6, as shown in FIG. 9.

In the Examples above, the change in absorbance of an agglutination mixture by light irradiation was determined; alternatively, the change in scattered light may be determined.

The present invention is practiced in the embodiments described above and demonstrates the following effects:

According to the present first invention, measurement time can be shortened, measurement costs can be reduced, and measurement operation can be simplified because a whole blood sample can be used directly without subjecting it to pretreatment

such as centrifugation, etc. Further, the opportunity for an operator to come into contact with blood can be decreased and the danger of infection can be significantly reduced.

According to the present second invention, accurate data can be obtained by conducting hematocrit correction.

CLAIMS

What Is Claimed Is:

1. An immunoassay method in which antibodies or antigens in a sample are subjected to agglutination reaction with insoluble carriers onto which antigens or antibodies specifically reacting with the antibodies or antigens in the sample have been immobilized and the resulting agglutination mixture is determined for the change in its absorbance or in its scattered light by irradiation with light, wherein said sample is whole blood and the whole blood is forcibly lyzed.

2. The immunoassay method according to claim 1, wherein whole blood is forcibly lyzed by mixing the whole blood with a low osmotic solution.

3. The immunoassay method according to claim 1, wherein whole blood is forcibly lyzed by mixing the whole blood with a solution of saponins for hemolysis.

4. The immunoassay method according to claim 1, wherein whole blood is forcibly lyzed by freezing and thawing the whole blood.

5. The immunoassay method according to claim 1, wherein whole blood is forcibly lyzed by ultrasonicating the whole blood.

6. The immunoassay method according to claim 1, wherein saponins for hemolysis are incorporated into an insoluble particle suspension reagent onto which antibodies or antigens specifically reacting with antigens or antibodies have been immobilized.

7. An immunoassay method, comprising the step of subjecting antibodies or antigens in whole blood as a sample to agglutination reaction with an insoluble particle suspension reagent onto which antigens or antibodies specifically reacting with the antibodies or antigens in the whole blood have been immobilized, the step of determining the resulting agglutination mixture for the change in its absorbance or in its

scattered light by irradiation with light, and the step of calculating the hematocrit % of as follows by the sample:

$$A' = A \times 100/(100 - \text{hematocrit \%})$$

where A is the absorbance or its change or the strength of light scattering or its change determined, and A' is the corrected absorbance or its change or the strength of light scattering or its change assuming that the plasma component in the sample is 100%.

ABSTRACT OF THE DISCLOSURE

The present invention provides an immunoassay method in which blood can be measured even without pretreatment by means of a centrifuge etc. In the present invention, antibodies or antigens in a sample are subjected to agglutination reaction with insoluble carriers onto which antigens or antibodies specifically reacting with the antibodies or antigens in the sample have been immobilized and the resulting agglutination mixture is determined for the change in its absorbance or in its scattered light by irradiation with light, wherein said sample is whole blood and the whole blood is forcibly lyzed.

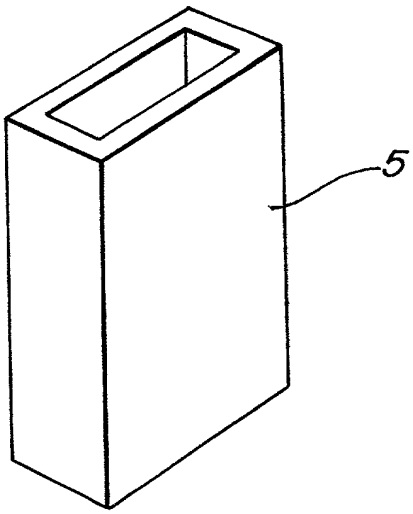


FIG. 1

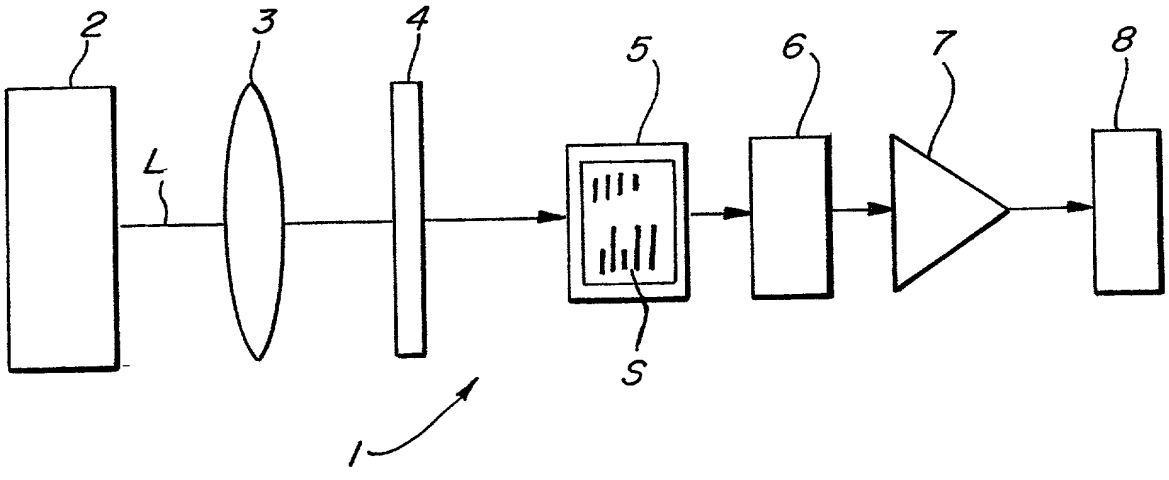


FIG. 2

FIG. 3

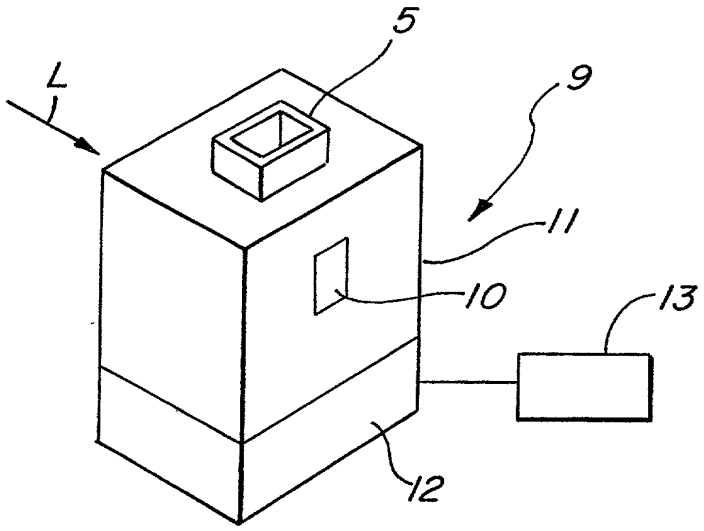
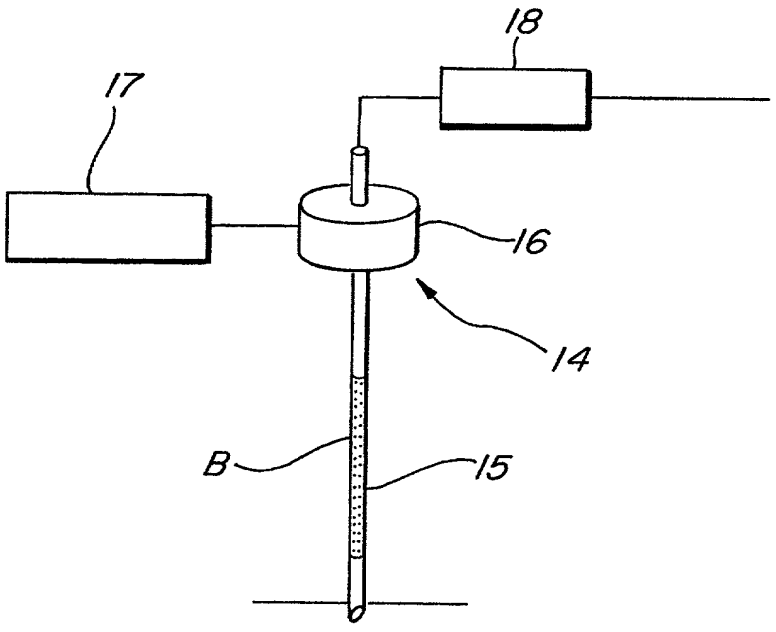


FIG. 4



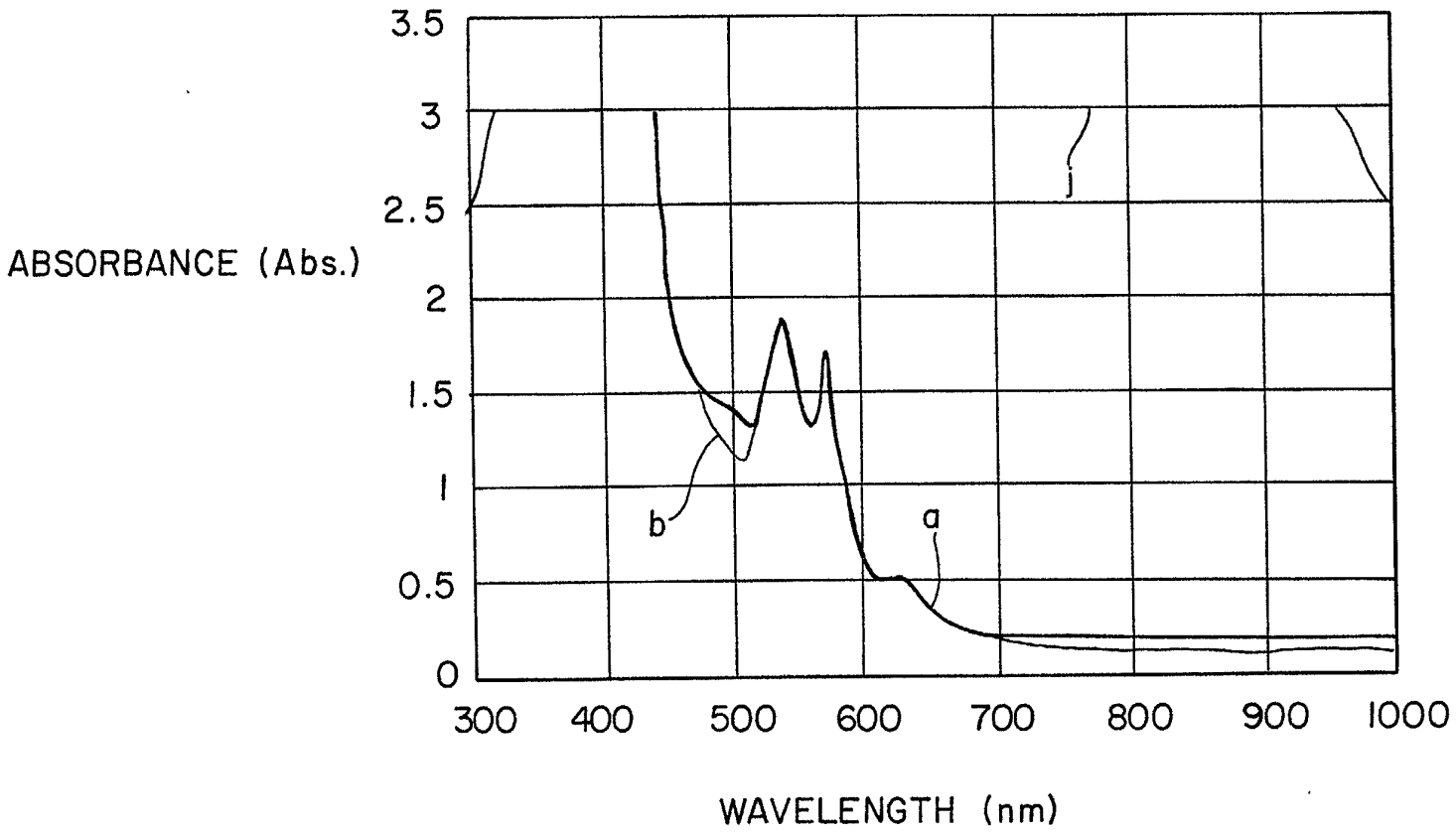


FIG. 5

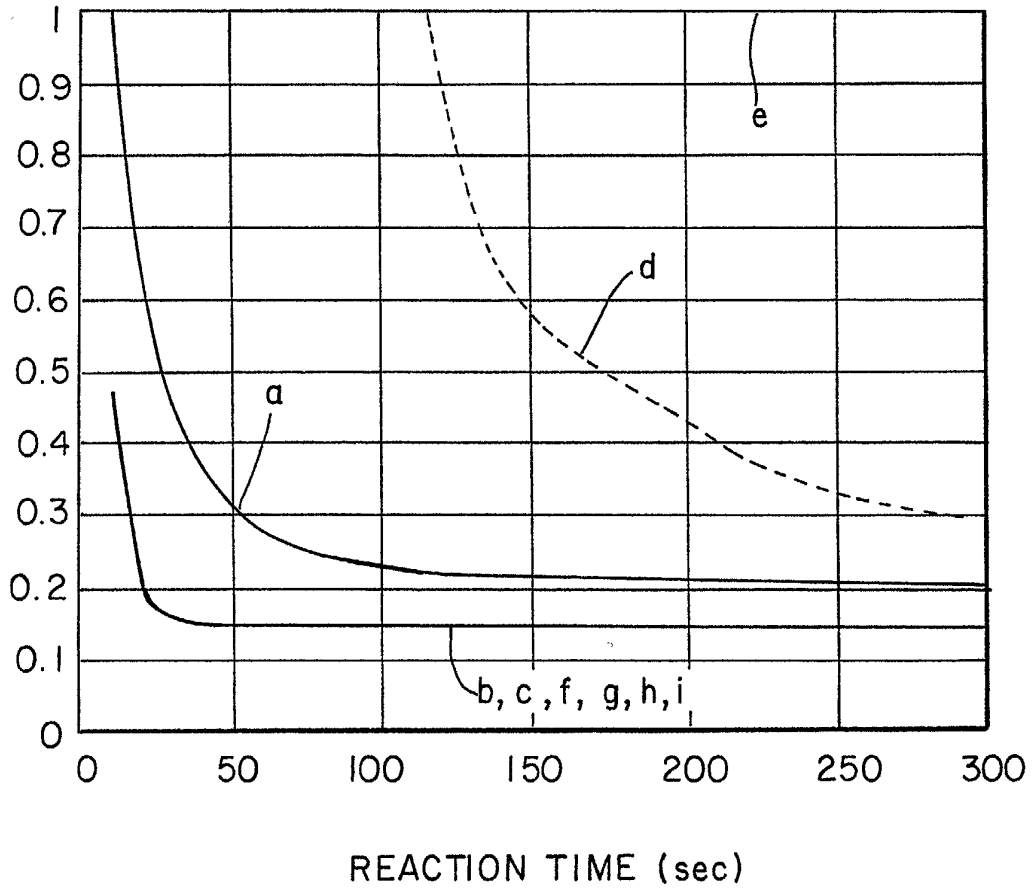


FIG. 6

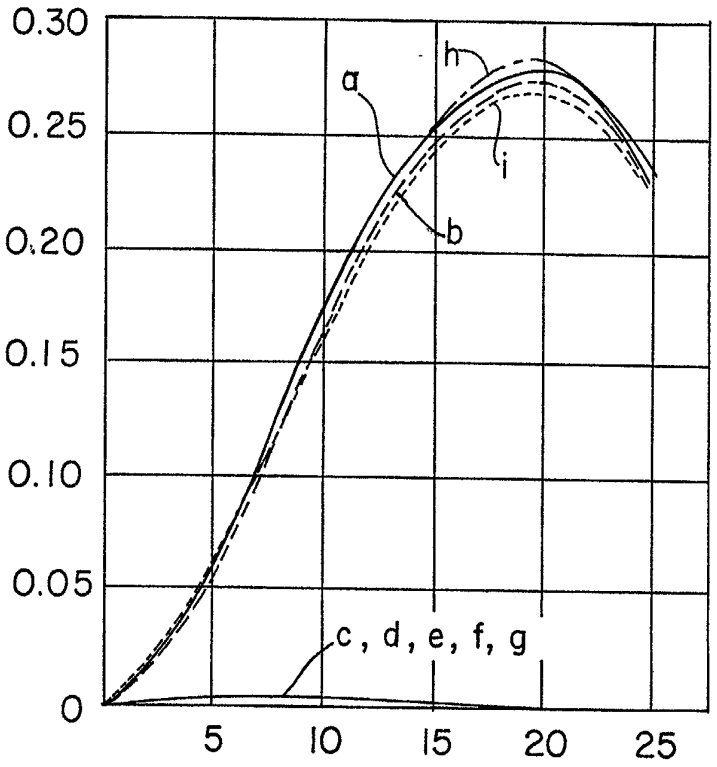


FIG. 7

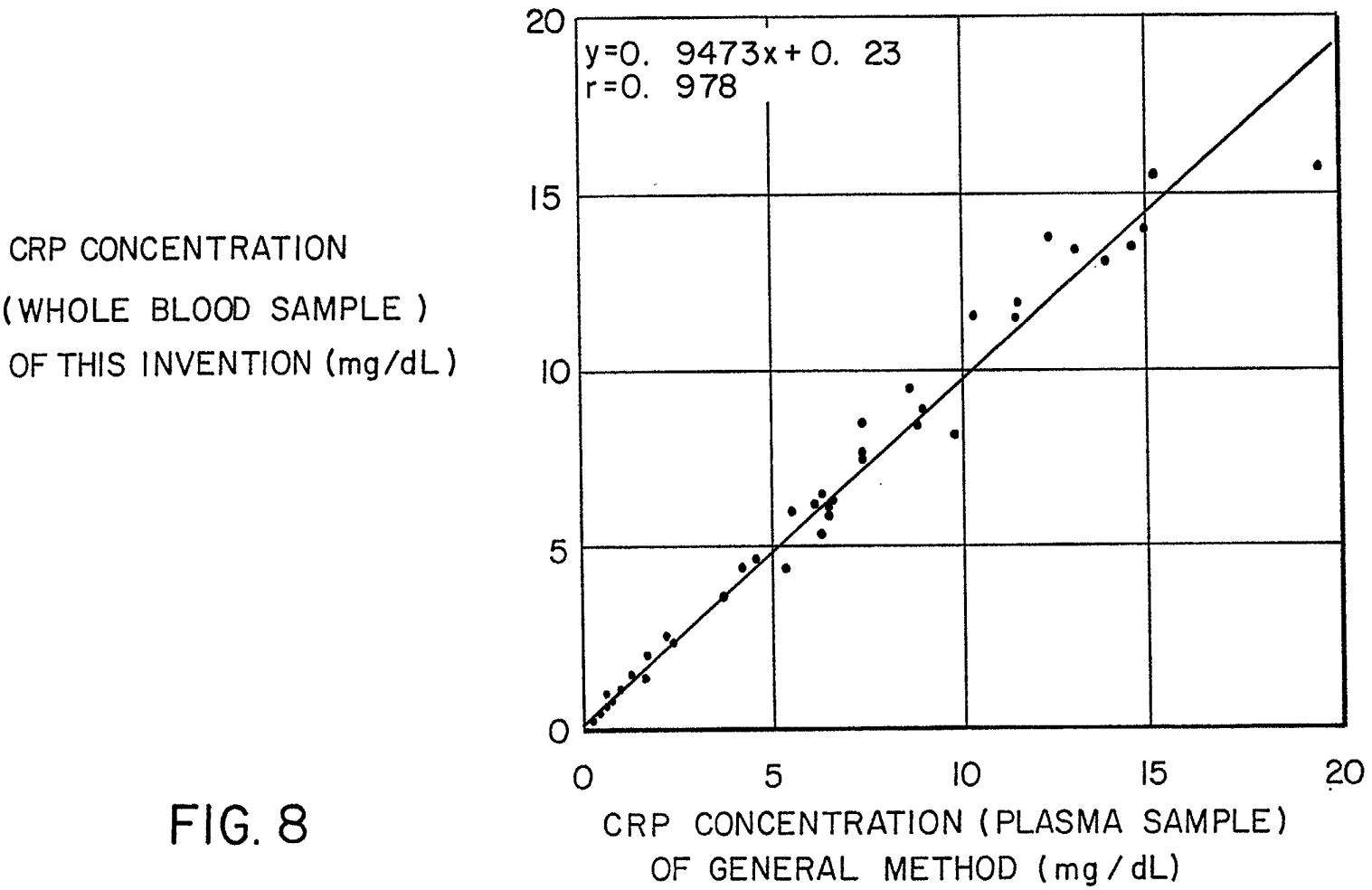


FIG. 8

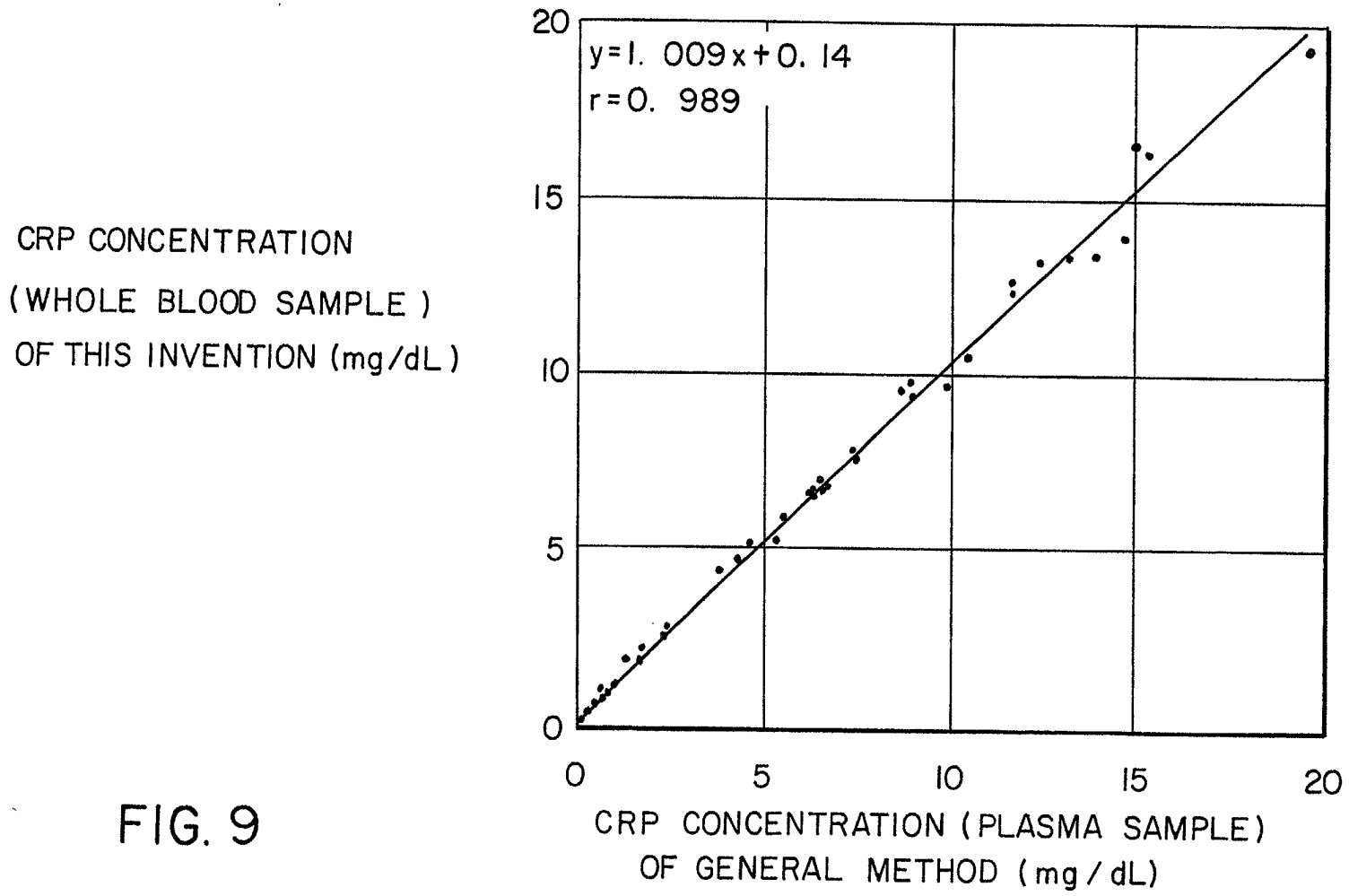


FIG. 9

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FUJ2-AZ72

COMBINED DECLARATION AND POWER OF ATTORNEY IN ORIGINAL APPLICATION

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that I verily believe that I am the original, first and sole inventor (if only one name is listed below) or joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought, entitled:

IMMUNOASSAY METHOD FOR LYZED WHOLE BLOOD

the specification of which:

(check one)

☐ is attached hereto.

☒ was filed on July 28, 1997 as

Application Serial No. 08/914,039

and was amended on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Foreign application(s), if any, for Patent or Inventor's Certificate Filed Within 12 Months Prior to the Filing Date of This Application:

Country	Application No.	Date of Filing (day, month, year)	Priority Claimed Under 35 U.S.C. 119
Japan	8-217966	30 July 1996	Yes x No
			Yes No
			Yes No
			Yes No
			Yes No
			Yes No

All Foreign Applications, if any, for Patent or Inventor's Certificate Filed More Than 12 Months Prior to the Filing Date of This Application:

Country	Application No.	Date of Filing

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in a manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. 1001) and may jeopardize the validity of the application or any patent issued thereon.

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POST OFFICE ADDRESS		
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RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		
FULL NAME OF FIFTH JOINT INVENTOR, IF ANY	INVENTOR'S SIGNATURE	DATE
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POST OFFICE ADDRESS		
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RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		